VPI/02-135 US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Examiner	:	Kevin E. Weddington
Group	:	1614
Applicants	:	Jugnu Jain-Pandey et al.
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Cambridge, Massachusetts
May 8, 2009

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF JUGNU JAIN UNDER 37 C.F.R. § 1.132

I, Jugnu Jain, do hereby declare:

1. I am a named inventor on the above-identified patent application (hereinafter the '114 Application).

2. I received a B.S. in Biology from Delhi University of Delhi, India, in 1982. In 1989 I received a Ph.D. in Molecular Genetics from the Plant Breeding Institute of Cambridge, England. After receiving my Ph.D., I was a Post-Doctoral Fellow at the Dana Farber Cancer Institute in Boston, Massachusetts from 1989-1992. From 1992 to 1996, I was employed as an Instructor at the Dana Farber Cancer Institute and Harvard Medical School. I have

published 27 papers in peer-reviewed journals including Nature and Science. A copy of my curriculum is attached as Exhibit 1.

3. In 1996, I joined Vertex Pharmaceuticals, Inc. (hereinafter "Vertex"). From 1996 to the present, I have been involved in several drug discovery projects at Vertex, with particular expertise in inflammation, neuroinflammation, immunosuppression and cancer. I have contributed to early stage target identification, validation and drug discovery to generation of structure activity relationships (hereinafter "SAR"), profiling of lead compounds, proof of concept (hereinafter "POC") in animal models, biomarker development, preclinical toxicity testing, Investigational New Drug (hereinafter "IND") filing and early clinical trials. I am familiar with most pharmaceutical aspects of drug development from research to clinical trials and I have diverse experience in the molecular, cellular, biochemical and animal pharmacology phases of drug development.

4. I am familiar with the November 12, 2008 Office Action (hereinafter "Office Action") in the above-identified application. I understand that, in the Examiner's view, claims 1, 2, 4-6, 10, 11 and 22 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Montgomery et al. (hereinafter "Montgomery") in US patent No. 4,210,745 in view of Stamos et al. (hereinafter "Stamos") in PCT publication number WO 00/56331. I also understand that in the Examiner's view claims 1, 2, 4-6, 10, 11 and 22 are provisionally rejected on the grounds of nonstatutory obviousness-type double patenting, as being unpatentable over claims 1-14 of U.S. Patent No. 6,498,178 (hereinafter "the '178 patent").

5. I make this declaration to demonstrate the surprising and unexpected synergistic activity of the combination of compound 181 with the anti-cancer agent fludarabine disclosed in the '114 Application.

6. I provide below in this declaration the following:

a) A detailed explanation of the graphical data in Figure 1 demonstrating the synergistic apoptotic effect observed when Daudi cells (a human hematological cancer cell line) were treated with four different concentrations of fludarabine alone, compound 181 alone, and fludarabine and compound 181 together in a 1:1 ratio. See, *infra* ¶ 7.

b) A detailed explanation of the graphical data in Figure 2 demonstrating the unexpected and strong synergistic apoptotic effect at 1μM and 10μM concentrations of the

combination of compound 181 and fludarabine on Daudi cells observed in the form of a Combination Index (hereinafter "CI"). See, *infra* ¶ 8.

7. Figure 1 (as filed and reproduced below) plots the percent apoptosis of Daudi cells versus concentration of compound 181 alone, fludarabine alone, the combination and no drug:

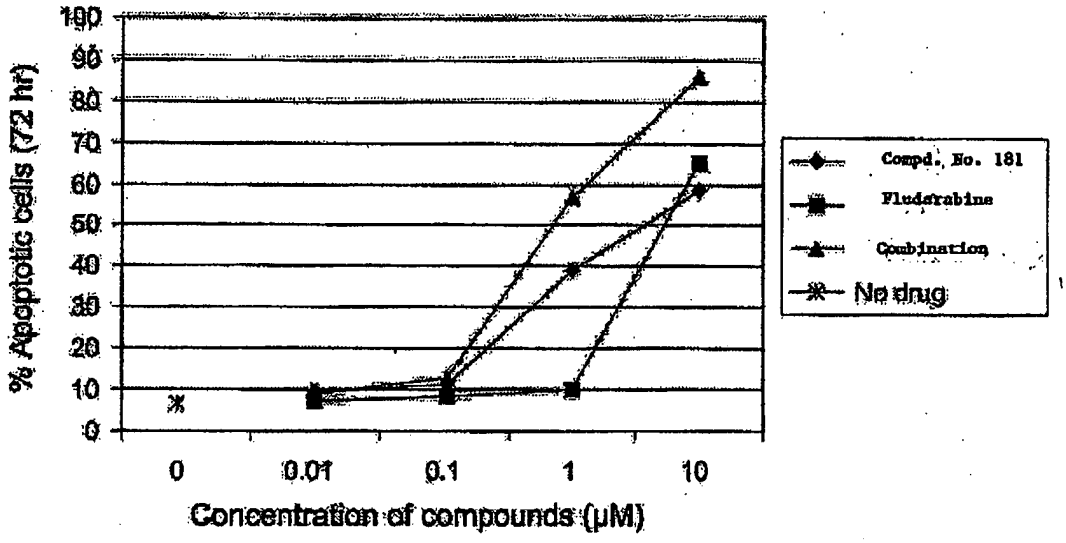

FIGURE 1: Plot of % Apoptosis versus concentration of Compd. 181, Fludarabine alone, the combination, and no drug

Figure 1 demonstrates the synergistic effect of combining a compound of formula (A) (here compound 181) with fludarabine versus the effect of the compound 181 alone and fludarabine alone and shows the percentage of cells in which apoptosis was induced. As described in the specification (e.g., see pages 58-59), Daudi cells were treated with concentrations of 0.01μM, 0. 1μM, 1μM and 10μM, of fludarabine alone, compound 181 alone, and fludarabine and compound 181 together in a 1:1 ratio.

In a concentration of up to 1μM, it can be seen that fludarabine alone induces apoptosis in only about 8-10% of cells. At a concentration of 10μM, there is strong increase in apoptosis to about 65%.

Compound 181 alone induces apoptosis in about 9-11% of cells up to concentrations of 0.1μM. At a concentration of 1μM there is an increase in apoptosis to about 39%, and in a concentration of 10μM apoptosis is induced in about 58% of cells.

In a concentration of 0.01μM or 0.1μM the combination of fludarabine and compound 181 induces apoptosis in about 9-13% of cells. At a 1μM concentration the combination induces apoptosis in almost 60% of the cells, and at a 10μM concentration, induces apoptosis in over 86% of cells.

Thus, Figure 1 clearly shows the synergistic effect of combining fludarabine and compound 181.

8. Figure 2 (as filed and reproduced below) shows the fraction of the Daudi cells affected by treatment with the combination of compound 181 and fludarabine. The four points plotted in Figure 2 (e.g., reading the four points from left to right on the x axis the concentrations, respectively are 0.01μM, 0.1μM, 1.0μM and 10μM) correspond to the concentrations of the combination plotted in Figure 1:

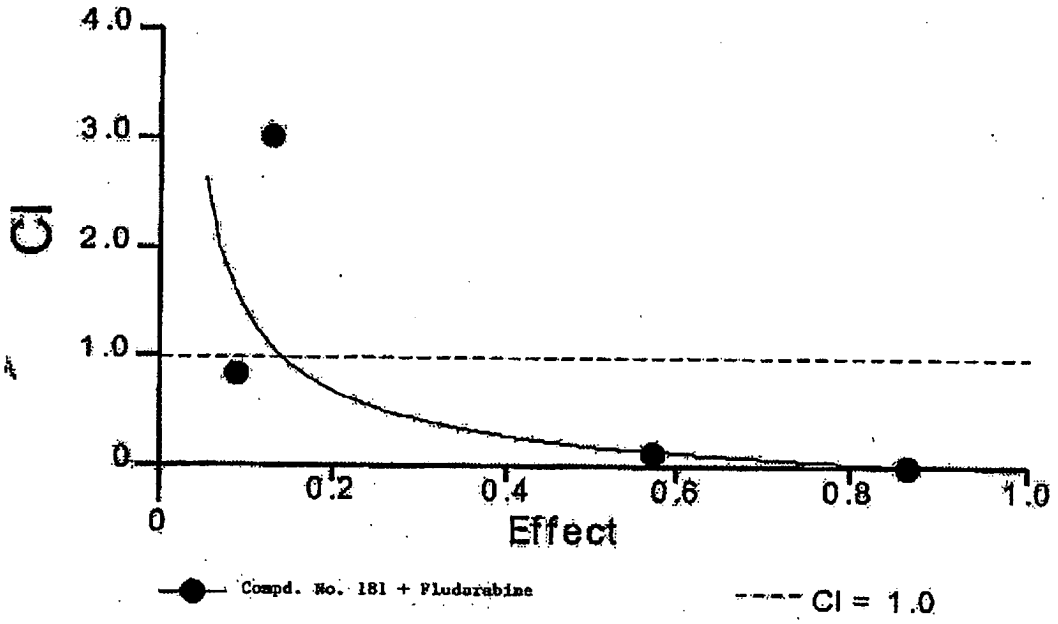

FIGURE 2: Plot of Effect versus Combination Index ("CI")

Figure 2 shows that the combination of compound 181 together with fludarabine has a strong synergistic effect. It can be seen that the apoptosis effect on the cancer cells is far greater than would be expected to result from this combination.

In this study on Daudi cells, compound 181 and fludarabine were used in a 1:1 ratio at the following concentrations:

Concentrations of both compound 181 and fludarabine	0.01μM	0.1μM	1μM	10μM
Fraction affected	0.087	0.127	0.57	0.863
Fraction affected (%)	8.7	12.7	57.0	86.3

In Figure 2, a dotted line is shown at the Combination Index or "CI" value of 1.0. The Combination Index or "CI" value shown in Figure 2 is a measure of the synergistic effect. If a combination a value of 1.0, this value represents that the effect of the components of the combination is additive. If the value is below 1.0, the effect of the components of the combination is additive. If the value is above 1.0, the effect is antagonistic, and consequently, the components should not be combined in order to achieve a better result. If the value is below 1.0, a synergistic effect is achieved by combining the compounds. Strong synergy can clearly be seen for the combinations at a 1μM and 10μM concentration by reference to the y-axis.

The Fraction affected represents the proportion, or percentage, of cells which underwent apoptosis. For example, at a concentration of 1μM of both compound 181 and fludarabine, 57% of cells underwent apoptosis. These values, shown in the Table above, are the x-axis values plotted in Figure 2. For the Fraction affected, the "Effective Dose" value may be calculated using appropriate software. For example, the ED50 value is the dose at which 50% of treated cells undergo apoptosis. Similarly, the ED75 and ED90 values (e.g., where 75% or 90%, respectively, of the cells undergo apoptosis) are also obtained through software calculations.

The Combination Index values at effective dose levels ED50, ED75 and ED90 (50%, 75% or 90% of the cells, respectively, undergo apoptosis) for a 1:1 combination of compound 181 and fludarabine were as follows in the Table below. The combination shows strong synergism, because the values are significantly below 1.

	Combination Index Values at			Description
Drug	ED50	ED75	ED90	
Cmpd 181 + Fludarabine (1:1)	0.21	0.08	0.03	Strong Synergism

Thus is can clearly be seen that a strong and unexpected synergistic effect is produced by combining the anti-metabolite fludarabine with compound 181 of the present invention.

9. I further declare that all statements made herein of my own knowledge are true and that all statements made herein on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code; and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

By: _____
Jugnu Jain, Ph.D.

Signed this 8th day of May, 2009
At Cambridge, MA, USA.